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EVALUATION OF CHANGES IN OXIDATIVE STRESS MARKER AND PROTEASE ACTIVITY IN WHEAT (*T. aestivum* L.) SEEDLINGS IN RESPONSE TO CARBENDAZIM FUNGICIDE

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ABSTRACT

Whether fungicide can exert some harmful effect by inducing oxidative stress apart from enhancing growth is a matter of concern. So the present study was undertaken with the objective of knowing the effect of fungicide on growth parameters and also to know about oxidative stress which is caused due to presence of fungicide. Activity of one more enzyme alkaline protease was assayed to know the possible reason for enhancement of growth of seedlings. Fungicide used was carbendazim having trade name Bavistin and study was conducted using wheat seedling. The results showed that growth parameters i.e. root and shoot length get increased due to presence of fungicide. Activity of alkaline protease was also found to be increased which may favor growth of seedlings. MDA and peroxidase activity was also found to be increased which indicates increase in oxidative stress. The growth parameters were found to be positively correlated with oxidative stress parameters.It was concluded from the present study that presence of fungicide enhances the growth of seedlings but it also induces oxidative stress in seedlings which could be harmful for the plants. So fungicides should be used in limited amount or instead of using chemical fungicide, biological fungicides should be used

KEYWORDS: Malondialdehyde, Peroxidase, Alkaline Protease, Root Length, Shoot Length

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Original Article

INTRODUCTION

Today environmental factors are affecting the production of active oxygen species in plants and are causing oxidative stress. The environmental factors that are causing stress are numerous like increased UV-B radiation, water, high salinity, temperature extremes, mineral nutrient deficiency, metal toxicity, herbicides, fungicides, air pollutants etc. As the oxidative damage in plants is increased, plant's antioxidant defense system gets activated. Among the four major active oxygen species H_2O_2 and the hydroxyl radical are considered as the most active, toxic and destructive radicals (Bartosz, 1997). Apart from preventing yield loss and promoting growth, some fungicides have been shown to possess antiozonant properties (Mackay *et al.*,, 1987). The mechanism for production of active oxygen species may be either by direct involvement in radical production or by inhibition of biosynthetic pathways.

In the present study fungicide Carbendazim was used to know whether it exert any oxidative damage in wheat seedlings. Along with oxidative markers, growth parameters were also estimated and correlation between growth parameters and oxidative markers under influence of fungicide was also calculated.

MATERIALS AND METHODS

Healthy wheat seedlings were sterilized with 0.1% $HgCl_2$ and were grown for seven days using different fungicide solution for test and distill water for control. All the parameters were estimated using seven days old seedlings by the following methods-

- **Root Length and Shoot Length:** These were determined using centimeter scale (Kabir *et al.*, 2008).
- **Alkaline Protease Activity:** It was estimated using Folin lowry reagent (Davis *et al.*, 1953). The enzymatic activity was measured by determining the extent of hydrolysis of casein as substrate followed by determination of unaltered casein by the Lowry's method of protein estimation.
- **MDA:** The level of lipid peroxidation was measured by estimating MDA, a decomposition product of peroxidised polyunsaturated fatty acid component of membrane lipid, using thiobarbituric acid (TBA) as the reactive material following the method of Heath and Packer (Heath *et al.*, 1968).
- **Peroxidase Activity:** The enzyme activity was assayed using o-dianisidine as hydrogen donor and H_2O_2 as electron acceptor. The rate of formation of yellow orange colored dianisidine dehydrogenation product was a measure of POD activity and assayed spectrophotometrically at 430nm (Summer *et al.*, 1943).

RESULTS AND DISCUSSIONS

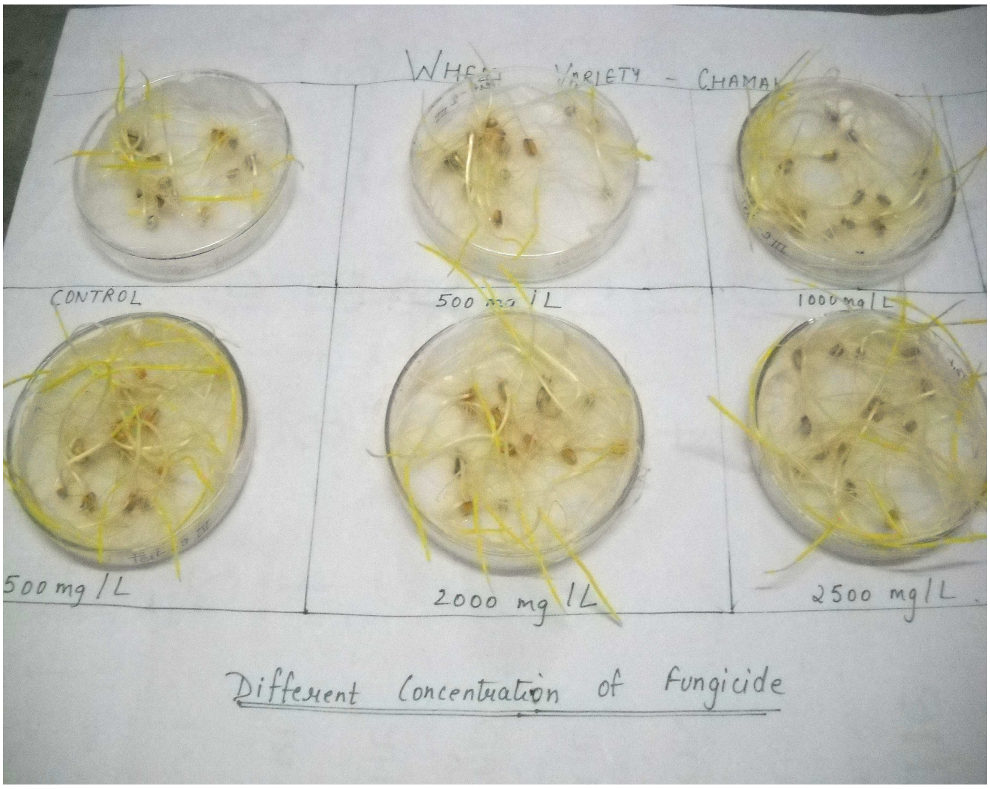

Figure 1: Seven Days Old Seedlings of Wheat (Variety Chamak) in Control Vs Treated with Concentration of Fungicide > 500 mg/L

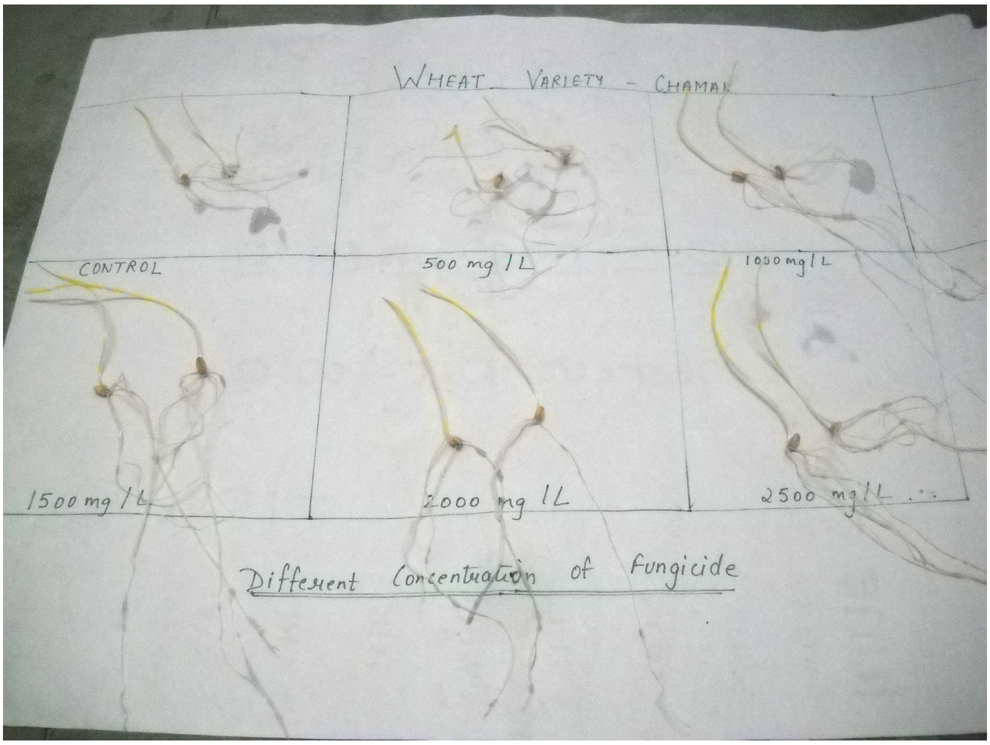

Figure 2: Effect of Concentration of Fungicide < 500 Mg/L on Visible Parameters (Root and Shoot Length) of Wheat Seedling

Table 1: Effect of Conc. of Fungicide (> 500 mg/L) on Various Parameters of Wheat Seedlings

S. No.	Conc. of Fungicide (mg/l)	MDA (mM/mg)	Peroxidase (Units/min/g)	Alkaline Protease (µmoles/h/mg Protein)	Root length (cm)	Shoot Length (cm)
1.	0	0.00096±0.00022	33.24±1.54	0.15±0.06	6.23±2.30	6.2±1.10
2.	500	0.0012 ± 0.000007*	35.34±0.02*	0.250±0.042*	10.0±2.32*	7.9±1.31*
3.	1000	0.0012 ± 0.000003*	35.02±1.09*	0.267±0.027*	10.3±2.30*	8.8±0.49**
4.	1500	0.00124 ± 0.000001*	36.03±0.02**	0.274±0.019**	11.4±0.28**	9.1±0.90**
5.	2000	0.00128 ± 0.000002*	36.44±0.01**	0.355±0.011**	11.2±0.70**	8.6±0.90**
6.	2500	0.00144 ± 0.000003**	37.03.±0.03**	0.378±0.022**	10.6±0.58**	8.5±0.45**

*= Values are Significant ($p<0.05$), **= Values are very significant ($p <0.01$) and ns= not significant ($p>0.05$).

Table 2: Showing % Increase in Studied Parameters of Wheat Seedlings under Influence of Conc. of Fungicide (> 500 mg/L)

S. No.	Conc. of Fungicide (mg/l)	MDA Content	Peroxidase Activity	Alkaline Protease Activity	Root Length	Shoot Length
1.	500	25.0%	6.31%	66.66%	60.51%	27.41%
2.	1000	25.0%	5.35%	78%	65.32%	42.41%
3.	1500	29.16%	8.39%	82.66%	82.98%	47.25%
4.	2000	33.33%	9.62%	136.66%	79.77%	39.19%
5.	2500	50.0%	11.40%	152%	70.14%	37.58%

Table 3: Showing Correlation between Growth Parameters and Oxidative Stress Markers

S. no.	Parameters	Root length	Shoot length	Alkaline Protease Activity
1	MDA	0.47	0.44	0.79
2	Peroxidase	0.88	0.80	0.96

As shown in table 1 root length of wheat seedling in control was 6.23 ± 2.3 cm. Root length of wheat seedlings at 500, 1000, 1500, 2000 and 2500 mg/L was found to be 10.0 ± 2.32, 10.3 ± 2.3, 11.4 ± 0.28, 11.2 ± 0.70 and 10.6 ± 0.58 cm respectively. The % increase in root length at 500, 1000, 1500, 2000 and 2500 mg/L concentration of fungicide was 60.51%, 65.32%, 82.98%, 79.77% and 70.14% respectively as compared to control. The highest % increase in root length was 82.98% which corresponds to 1500 mg/L of fungicide.

In control shoot length was observed to be 6.2 ± 1.10 cm as shown in table 1. Shoot length at 500, 1000, 1500, 2000 and 2500 mg/L concentration of fungicide was found to be 7.9 ±1.31, 8.83 ± 0.49, 9.13 ± 0.90, 8.63 ± 0.90 and 8.53 ± 0.45 cm respectively. The % increase at 500, 1000, 1500, 2000 and 2500 mg/L concentration of fungicide was found to be 27.41%, 42.41%, 47.25%, 39.19% and 37.58% respectively. The highest increase 47.25% was found at 1500 mg/L concentration of fungicide.

Alkaline protease activity of wheat seedling in control was 0.15 ± 0.06 µmoles/h/mg protein. As shown in table 1 alkaline protease activity of wheat seedlings at 500, 1000, 1500, 2000 and 2500 mg/L was found to be 0.250±0.042, 0.267±0.027, 0.274±0.019, 0.355±0.011 and 0.378±0.022 µmoles/h/mg protein respectively. The % increase in alkaline protease activity at 500, 1000, 1500, 2000 and 2500 mg/L concentration of fungicide was 66.66%, 78%, 82.66%, 136.66% and 152%respectively as compared to control. The highest % increase in alkaline protease activity was 152% which corresponds to 2500 mg/L of fungicide.

As shown in table 1 MDA content of wheat seedling in control was 0.00096±0.00022 mM/mg. MDA content of wheat seedlings at 500, 1000, 1500, 2000 and 2500 mg/L was found to be 0.0012 ± 0.000007, 0.0012 ± 0.000003, 0.00124 ± 0.000001, 0.00128 ± 0.000002 and 0.00144 ± 0.000003 mM/mg respectively. The % increase in MDA content at 500, 1000, 1500, 2000 and 2500 mg/L concentration of fungicide was 25%, 25%, 29.16%, 33.33% and 50% respectively as compared to control. The highest % increase in MDA content was 50% which corresponds to 2500 mg/L of fungicide.

Peroxidase activity of wheat seedling in control was 33.24±1.54 units/min/g as shown in table 1. Peroxidase activity of wheat seedlings at 500, 1000, 1500, 2000 and 2500 mg/L was found to be 35.34±0.02, 35.02±1.09, 36.03±0.02, 36.44±0.01 and 37.03.±0.03 units/ min/g respectively. The % increase in peroxidase activity at 500, 1000, 1500, 2000 and 2500 mg/L concentration of fungicide was 6.31%, 5.35%, 8.39%, 9.62% and 11.40% respectively as compared to control. The highest % increase in peroxidase activity was 11.40% which corresponds to 2500 mg/L of fungicide.

The results demonstrated that root length was increased with increasing concentration of fungicide. Results were in agreement with the work done by various scientists (Sarkar and Saxena, 2005).

Shoot length gets increased with increase in concentration of fungicide. Our results were contrary to the work which reported that systemic fungicides which are based on SBI (sterol biosynthesis inhibitor) are closely related to plant growth regulators the use of which at higher than labeled rates shorten the internodes which may lead to slow shoot growth (Windham and Windham, 2004).

Alkaline protease activity which is a marker for degradation of protein was also found to be increased with increase in concentration of fungicide. The work which shows decrease in protease activity by endosulfan treatment was not parallel to our result (Vidhyasagar *et al.,* 2009).

The present study suggested increase in MDA content of seedlings treated with fungicide. Thus lipid peroxidation measured in terms of MDA increases with increase in concentration of fungicide. The results were supported by the study

which showed positive correlation between MDA and concentration of fungicide (Liu *et al.,* 2005). Results were not similar to work of scientist who reported that fungicide treatments to wheat plants at growth stage decrease MDA content (Zhang *et al.,* 2010).

Peroxidase content of wheat seedlings gets increased with increase in concentration of fungicide. Lipid peroxidation mediated by activated toxic oxygen species should be accompanied by changes in activities of enzymes involved in oxygen metabolism (Velikova *et al.*, 2000). Peroxidase is one of the major systems for the enzymatic removal of H_2O_2 in plants. The increased activity of peroxidase in plants suggests the protective role of the enzyme in different stress situations, such as acid rain (Velikova *et al.,* 2000), Al toxicity (Cakmak and Horst, 1991) etc. Moreover, azoxystrobin and epoxiconazole fungicides induced a delay of senescence of *Triticum aestivum* mainly due to an enhancement of the antioxidative potential protecting the plants from harmful active oxygen species (Wu and Von, 2002). The increase in peroxidase activity may be due to the metabolic response to environmental stress (Fang and Kao, 2000). Modulations in antioxidant system could probably be the result of Artea/Punch-induced toxicity which could lead to an oxidative stress status (Bensoltan *et al.*, 2006). Increase in peroxidase activity by exogenous application of penconazole to drought-stressed plants (Hassanpour *et al.,* 2012).

From the present study it was evident that fungicide induces oxidative stress thereby increasing lipid peroxidation in growing seedlings and that peroxidase enzyme may serve as important defensive antioxidants to combat fungicide induced oxidative damage. At higher concentration of fungicide the increase in root and shoot length of seedlings may be due increased availability of amino acids for protein synthesis. These amino acids may result from increased activity of alkaline protease enzyme hydrolyzing seed protein. Positive correlation was seen between the growth parameters and oxidative stress parameters at all concentration of fungicide. Thus apart from accelerating growth of seedlings, fungicide also induces oxidative stress which may be one of the drawbacks of using fungicide.

CONCLUSIONS

It can be concluded from the present study that fungicides are responsible for causing oxidative stress in growing seedlings which is one of the important drawback of using fungicides. So new fungicides should be designed which prevents yield loss without causing any harm to the crop.

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